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EFFECT OF SUBLETHAL X-IRRADIATION OF GUINEA PIGS ON VACCINAL TULAREMIA INFECTION

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EFFECT OF SUBLETHAL X-IRRADIATION OF GUINEA PIGS ON VACCINAL TULAREMIA INFECTION

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ABSTRACT

To delineate differences in the resistance of irradiated and nonirradiated animals to live tularemia vaccine, the chronological appearance and growth rate of LVS in the lung, liver, spleen, and blood of guinea pigs were studied. Nonirradiated controls and guinea pigs having received 140 R three days previously were exposed via the respiratory route to 10^6 cells of LVS and sacrificed at intervals from one to 21 days. No major differences were noted in the time of appearance, growth rate, maximum organism content, or time of clearance of LVS from the tissues of irradiated and nonirradiated animals; hence, there was no evidence of a change from a self-limiting to a fulminating type of infection resulting from irradiation of the animals. Also, no appreciable difference in the time of appearance of agglutinins or maximal titer was noted in the two groups of animals. The production of agglutinins in irradiated animals in response to vaccination with LVS is in contrast to the reported inhibition of antibody response in animals inoculated with killed organisms or purified antigens subsequent to irradiation.

EFFECT OF SUBLETHAL X-IRRADIATION OF GUINEA PIGS ON VACCINAL TULAREMIA INFECTION

We previously reported that sublethal X-irradiation of guinea pigs three days prior to respiratory exposure to normally innocuous doses of Pasteurella tularensis vaccine strain LVS resulted in 25 per cent mortality.* No differences were noted between irradiated and non-irradiated animals with regard to febrile, hematological, serological, or immune responses. The present study was designed to elucidate any difference in the growth rate of LVS in the tissues of irradiated and nonirradiated animals. In addition, the appearance of serum agglutinins was determined.

A 1000-KVP X-ray unit was used for irradiation. Whole body exposures were made in a plastic wheel cage at a distance of 100 cm; dose rates ranged from 56 to 73 roentgens (R) per minute. Total dose delivered to the animals was 140 R, the maximum sublethal dose for 325- to 375-g, male, Hartley strain, guinea pigs used as the test animal. Three days following whole body irradiation, groups of irradiated and nonirradiated guinea pigs were permitted to inhale 10^6 viable cells of vaccine strain (LVS) contained in a small particle aerosol. Four irradiated and four nonirradiated animals were sacrificed at eight intervals ranging from one to 21 days following aerosol exposure. Blood was collected for serological and cultural studies and the left lung, spleen, and a liver lobe were removed aseptically. The solid tissues were weighed, moistened with gel-saline, and then ground in Ten Broeck homogenizers. Appropriate dilutions were prepared from all tissues and plated on glucose cysteine blood agar and incubated four days at 37°C. This technique allowed the calculation of the number of viable LVS cells per g of tissue.

Comparable groups of animals were not sacrificed but used to obtain data on survival and subsequent immunity.

The growth curves of LVS in the lungs of irradiated and nonirradiated animals are shown in Figure 1. Each point in this and the following figures represents the average viable count of P. tularensis per g of tissue and was based solely on samples from which the organism was recovered. Based on impinger recovery the inhaled dose of LVS per g of lung tissue should have been approximately 4.0×10^4 cells. It was estimated that approximately 10 per cent of the dose would be retained. If the curve is extrapolated to time zero, the value would be approximately 4.0×10^3, precisely the predicted retained dose. No appreciable difference in the growth rate of LVS was observed in the lungs of irradiated and nonirradiated animals. A logarithmic increase in viable population

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* Nutter, J.E., and Eigelsbach, H.T. "Exposure of guinea pigs to X-irradiation and P. tularensis of reduced virulence," Medical Bacteriology Division, U.S. Army Biological Laboratories, Frederick, Maryland. September 1964. (Technical Manuscript 163)

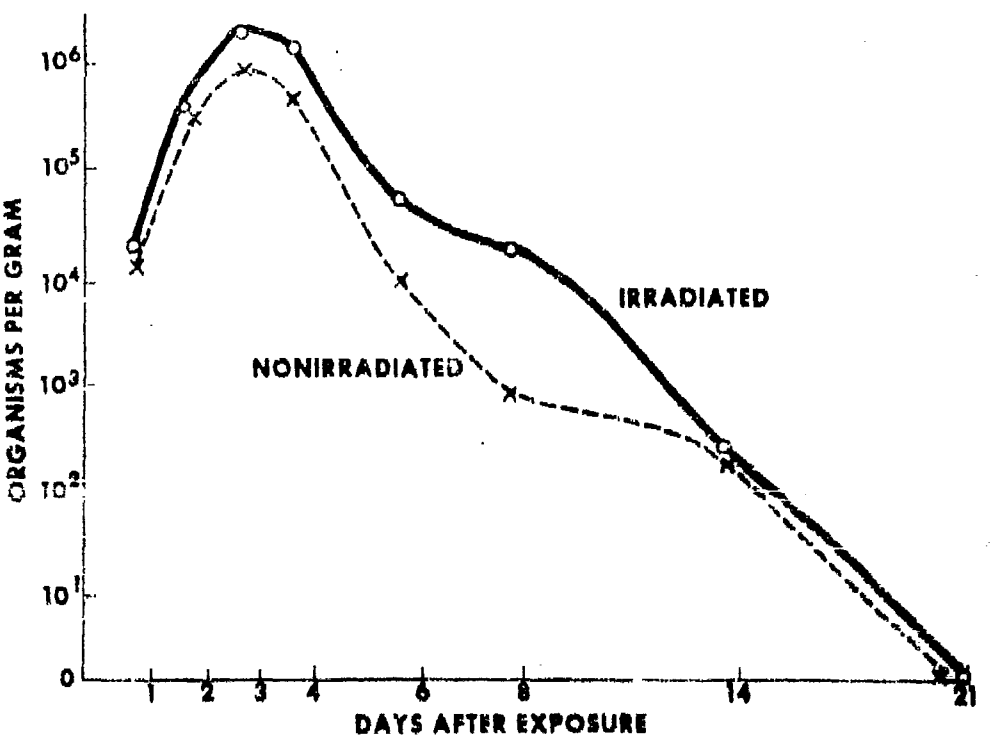

Figure 1. Growth of LVS in the Lung of Irradiated and Nonirradiated Guinea Pigs.

occurred from Day 1 to Day 3. Maximum counts per g of tissue were obtained at three days. Thereafter, the number of organisms gradually declined; none were recovered from the lungs of either group at 21 days. On Day 8, the lungs of the irradiated and nonirradiated animals contained approximately 10^4 and 10^3 viable cells per g, respectively. Since the lungs of irradiated and nonirradiated animals had previously contained a much greater number of cells and this difference occurred during clearance, it was postulated that a log difference in count at this time would not account for the increased lethality.

The growth curves of LVS in the liver are shown in Figure 2. In both irradiated and nonirradiated animals, the organisms first appeared two days after respiratory exposure and maximum growth occurred on the third day. From the first through the sixth day, the curves were similar. On Day 8, LVS was not isolated from nonirradiated animals whereas approximately 20 organisms were present per g of liver of irradiated animals. LVS was not isolated from the liver of either group on Days 14 and 21.

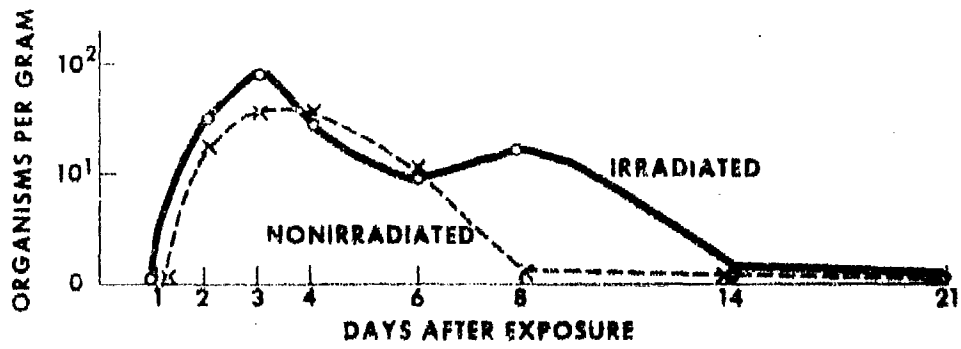

Figure 2. Growth of LVS in the Liver of Irradiated and Nonirradiated Guinea Pigs.

Figure 3 presents the growth pattern of LVS cells in the spleen of the two groups of animals. Organisms were first recovered on the second day after exposure. A logarithmic increase in LVS was evident from the first to the third day. With the possible exception of data obtained eight days after exposure to LVS, no difference in growth rate was observed between the irradiated and nonirradiated animals.

The maximum LVS population per entire spleen was only 500 organisms, far below the level expected in the case of an active or fulminating infection. After eight days viable counts gradually declined. LVS was not recovered from the spleens of animals of either group 21 days after respiratory exposure.

LVS was isolated only sporadically from the blood and no particular pattern was evident. Viable counts were of the order of five to ten organisms per ml.

The agglutinin response of irradiated and nonirradiated guinea pigs is shown in Table I. Agglutinins were first observed in the sera of either group of animals eight days after exposure to LVS. The titers of all animals remained positive throughout the 30 day period. In general, agglutinin titers of irradiated animals were lower than agglutinin titers of nonirradiated animals.

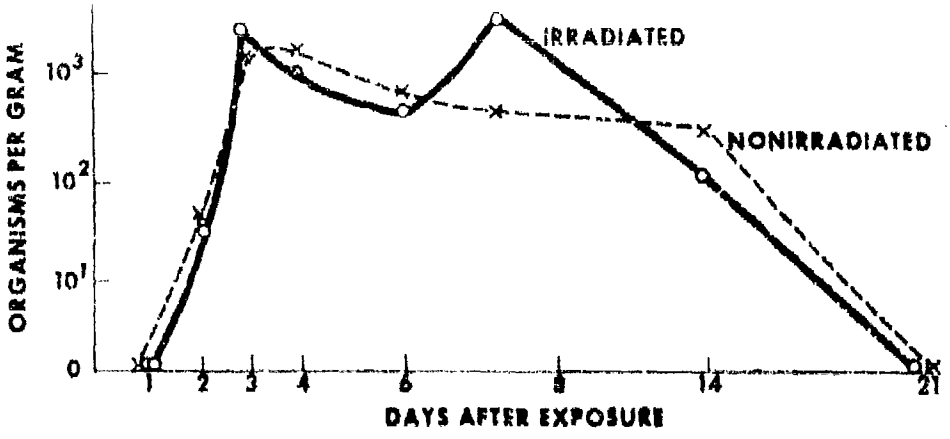

Figure 3. Growth of LVS in the Spleen of Irradiated and Nonirradiated Guinea Pigs.

TABLE I. AGGLUTININ TITERS OF GUINEA PIGS EXPOSED AEROGENICALLY TO FRANCISELLA TULARENSIS STRAIN LVS

Day After Exposure	Mode	
	Irradiated	Nonirradiated
1,2,3,4,6	0	0
8	1:20	1:80
14	1:320	1:320
21	1:160	1:320
30	1:160	1:640

Thirty days following the aerosol exposure to the vaccine strain, 23 per cent of the irradiated vaccinated group held for observation had died. Few deaths occurred in vaccinated or irradiated controls. Animals of each group were challenged subcutaneously with 10^3 cells of highly virulent F. tularensis SCNU 84. All nonvaccinated animals, irradiated and nonirradiated, succumbed within six days. Vaccinated animals, both irradiated and nonirradiated showed resistance to the challenge. However, nonirradiated vaccinees exhibited a slightly higher grade immunity than irradiated vaccinees. The difference in immunity was not as marked as that reported in the literature for other microorganisms which describes the poor immune response of irradiated animals to killed vaccines in comparison to nonirradiated animals.

In general, no marked differences were noted in the growth rate, maximal viable population, time of appearance, and clearance of LVS in the blood, lung, liver, and spleen of irradiated and nonirradiated animals. All irradiated as well as nonirradiated animals produced agglutinins. Only a slight suppression in the resistance to virulent challenge was observed among irradiated vaccinees in comparison with nonirradiated vaccinees. Therefore, the mortality response observed in sublethally irradiated guinea pigs exposed to normally innocuous doses of F. tularensis vaccine strain LVS is not correlated with a major defect in the immunological response of the animals to live vaccine. Also no evidence for a change from a self limiting to a fulminating type of infection was obtained.